IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Confirmation No.	:	**6518**
Appln. No.	:	10/534,660
Applicant	:	Guenter Harth, et al.
Filed	:	11/28/2005
TC/A.U.	:	1623
Examiner	:	Issac, Roy P.
Docket No.	:	1951326-00005 NAT
Customer No.	:	45200
Title	:	Anti-Microbial Agents Derived From Methionine Sulfoximine Analogues

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Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
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DECLARATION OF MARCUS A. HORWITZ, M.D. AND OWEN W. GRIFFITH, PH.D. UNDER 37 CFR §1.132

Commissioner for Patents
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We, Marcus A. Horwitz and Owen W. Griffith, declare the following:

1. We are inventors of the above-identified patent application, Serial No. 10/534,660.

2. We have read and are familiar with the Office Action mailed November 7, 2007 pertaining to this application.

3. We understand that in the Office Action mailed November 7, 2007, the Examiner rejected the claims under 35 USC §103(a) as being obvious over a combination of U.S. Patent No. 6,013,660, Griffith et al. (Methods in Enzymology, 143:286-291, 1987) and Harth et al. (J. Exp. Med. 189:1425-1435, 1999).

4. The Examiner has stated that since certain prior art compounds are selective inhibitors of mammalian glutamine synthetase (GS), that they should also be able to inhibit mycobacterial GS.

5. The prior art uses the term selective to indicate the selectivity of the compounds for glutamine synthetase relative to γ-glutamylcysteine synthetase. The prior art does not address selectivity between mammalian and mycobacterial forms of GS, and that was never tested prior to the studies resulting in the instant application.

6. Furthermore, mammalian and mycobacterial GS enzymes do not have significant sequence homology. Only 74 (15.5%) of the 478 amino acids of *M. tuberculosis* GS are identical to mammalian GS and when the total number of identical and similar residues are combined (identical + conservative changes) the percent homology is only 30.8% (Figure 1).

7. Additionally, mammalian GS is comprised of 8 subunits of the 373 amino acid sequence depicted in Figure 1 while mycobacterial GS is comprised of 12 subunits of the 478 amino acid sequence in Figure 1.

8. Therefore, the activity of a compound as an inhibitor against mammalian GS is not predictive of its activity against mycobacterial GS both because there is a lack of significant sequence homology and there are obvious structural differences.

9. The activity of different compounds against mycobacterial and mammalian GS and γ-glutamylcysteine synthetase are depicted in Table 1 (attached) which demonstrates that unlike L-methionine-S-sulfoximine (MSO), α-ethyl-MSO has the potential to inhibit mycobacterial GS while sparing human GS.

10. The activity of different compounds against sheep brain GS and rat kidney γ-glutamylcysteine synthetase are depicted in Table 2 (attached), which is based on data in Griffith and Meister (J. Biol. Chem. 253, 2333-2338 (1978)). Table 2 shows that at higher concentrations than used for the studies in Table 1, α-ethyl-MSO is able to almost completely inhibit the mammalian GS while sparing mammalian γ-glutamylcysteine synthetase.

11. MSO and α-methyl-MSO have similar potency as inhibitors of isolated mammalian GS and γ-glutamylcysteine synthetases (Tables 1 and 2), but were found by Griffith and Meister to have greatly different effects *in vivo*. Thus, L-SR-MSO reproducibly caused convulsions in mice at a dose of 1 mmol/kg whereas DL-SR-α-methyl-MSO required a dose of 16 mmol/kg to cause convulsions. No convulsions were seen at a dose of 8 mmol/kg. α-ethyl-MSO was not convulsive at 16 mmol/kg but caused convulsions in a minority of mice at a dose of 32 mmol/kg (J. Biol. Chem. 253, 2333-2338 (1978); see last paragraph p. 2335, continuing on to p. 2336). Griffith and Meister attributed the lower *in vivo* toxicity of α-methyl-MSO, which was not predicted from the *in vitro* inhibition studies, to lesser transport of α-methyl-MSO across the blood brain barrier compared with MSO. Based on the reported observations of Griffith and Meister, we were not able to predict in advance of the *in vivo* studies reported in the instant application whether or not α-methyl-MSO or α-ethyl-MSO would be effective inhibitors of mycobacterial GS *in vivo* even after we had shown that they were effective inhibitors of isolated mycobacterial GS.

12. The information in Tables 1 and 2 and the discussion in paragraph 11 together demonstrate that MSO, α-methyl-MSO and α-ethyl-MSO do not have similar or predictable ability to inhibit mycobacterial GS or various mammalian GS and γ-glutamylcysteine synthetases and they do not have similar or predictable effects *in vivo*.

13. All statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of

the United States Code, and such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

Inventor: Marcus A. Horwitz, M.D. Date May 5, 2008

Inventor: Owen W. Griffith, Ph.D. Date 5/6/08

Figure 1

Sequence 1 = *M. tuberculosis* glutamine synthetase
Sequence 2 = mammalian glutamine synthetase

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CLUSTAL 2.0.5 multiple sequence alignment

1 MTEKTPDDVFKLAKDEKVEYVDVRFCDLPGIMQHFTIPASAFDKSVFDDGLAFDGSSIRG 60
2 ---MTTSASSHLNKGIKQVYMSLPQGEKVQAMYIWIDGT--------GEGLRCKTRTLD- 48
      *..   :* *. *  *:.:   :    *  :   :         .:**  .  ::

1 FQSIHESDMLLLPDPETARIDPFRAAKTLNINFFVHDPFTLEPYSRDPRNIARKAENYLI 120
2 ---SEPKCVEELPEWNFDGSSTLQSEGSNSDMYLVPAAMFRDPFRKDP-NKLVLCEVFKY 104
      . . :  **: :    ..:::  : .  ::*  .:  :*: :** *    .* :

1 STGIADTAYFGAEAEFYIFDSVSFDSRANGSFYEVDAISGWWNTGAATEADGSPNRGYKV 180
2 NRRPAETNLR--HTCKRIMDMVSNQHPWFGMEQEYTLMG------TDGHPFGWPSNGFPG 156
  .   *:*     .:   *:* ** :    *  *   :.      :  .. * *..*:

1 RHKGGYFPVAPNDQY-VDLRDKMLTNLINSGFILEKGHHEVGSGGQAEINYQFNSLLHAA 239
2 PQGPYYCGVGADRAYGRDIVEAHYRACLYAGVKIAGTNAEVMP-AQWEFQIGPCEGISMG 215
  :   *  *..:  *  *: :      : :*. :   : ** . .* *::    . :  .

1 DDMQLYKYIIKNTAWQNGKTVTFMPKPLFGDNGSGMHCHQSLWKDGAPLMYDETGYAGLS 299
2 DHLWVARFILHRVCEDFGVIATFDPKPIPG-NWNGAGCHTNFSTK---AMREENGLKYIE 271
  *.: : ::*:::... : *  .** ***: * * .*  ** .: ..    * :*.*   :.

1 DTARHYIGGLLHHAPSLLAFTNPTVNSYKRLVPGYEAPINLVYSQR---NRSACVRIPIT 356
2 EAIEKLS---KRHQYHIRAYDPKGGLDNARRLTGFHETSNINDFSAGVANRSASIRIPRT 328
  :: .:      :*   : *:      .  * :.*:. . *:   .    ****.:*** *

1 GSNPKAKRLEFRSPDSSGNPYLAFSAMLMAGLDGIKNKIEPQAPVDKDLYELPPEEAASI 416
2 VGQEKKGYFEDRRPSANCDPFSVTEALIRT------------------------------ 358
  .: *   :* * *.:. :*: . .*:: :

1 PQTPTQLSDVIDRLEADHEYLTEGGVFTNDLIETWISFKRENEIEPVNIRPHPYEFALYY 476
2 ------------------------------------CLLNETGDEPFQYKN--------- 373
                                       .: .*.  **.: :

s
1 DV 478
2 --
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* = identical amino acid
: = conserved substitutions
. = semi-conserved substitutions

Table 1

		Glutamine synthetase activity remaining[%][5]		γ-Glutamylcysteine synthetase activity remaining [%][5]	
Inhibitor	Conc. [μM]	*M. tb.*	Human	*M. tb.*	Human
None	0	100	100	100	100
MSO[1]	2	15	87	96	94
MSO	200	5	59	38	42
α-methyl MSO[2]	2	13	92	98	92
α-methyl MSO	200	4	51	46	51
α-ethyl MSO[3]	2	8	99	95	98
α-ethyl MSO	200	2	97	82	93
BSO[4]	2	99	99	5	8
BSO	200	92	96	2	1

[1] L-methionine-*SR*-sulfoximine
[2] α-methyl-DL-methionine-*SR*-sulfoximine
[3] α-ethyl-DL-methionine-*SR*-sulfoximine
[4] L-buthionine-*SR*-sulfoximine
[5] GS activity was assayed in the transfer assay and γ-glutamylcysteine synthetase activity was assayed in the glutamate-aminobutyrate condensation assay. Results are expressed as % enzyme activity after incubation with inhibitors.
All inhibitors were mixtures of the stereoisomers as shown. For MSO the concentration of active inhibitory isomer was about 50% of that shown; for α-methyl-MSO and α-ethyl-MSO the concentration of active inhibitory isomer was about 25% of that shown.

Table 2

Inhibitor	Conc. [μM]	Sheep brain glutamine synthetase activity remaining [%][4]	Rat kidney γ-glutamylcysteine synthetase activity remaining [%][4]
None	0	100	100
MSO[1]	50	38	15
MSO	200	9	15
α-methyl MSO[2]	100	20	12
α-methyl MSO	400	4	12
α-ethyl MSO[3]	400	59	92
α-ethyl MSO	2,000	15	90
α-ethyl MSO	20,000	2	89

[1] L-methionine-*SR*-sulfoximine

[2] α-methyl-DL-methionine-*SR*-sulfoximine

[3] α-ethyl-DL-methionine-*SR*-sulfoximine

[4] Data is taken from Table 1 of O.W. Griffith and A. Meister, J. Biol. Chem. 253, 2333-2338 (1978); % inhibition as shown in the publication were converted to % activity remaining in the Table above. Glutamine synthetase and γ-glutamylcysteine synthetase were each incubated for 20 min in the presence of ATP and inhibitor, and were then assayed for remaining enzyme activity based on conversion of [^{14}C]glutamate to [^{14}C]glutamine (glutamine synthetase) or conversion of α-amino-[^{14}C]butyrate to γ-glutamyl-α-amino-[^{14}C]butyrate (γ-glutamylcysteine synthetase). All inhibitors were mixtures of the stereoisomers as shown. For MSO the concentration of active inhibitory isomer was about 50 % of that shown; for α-methyl-MSO and α-ethyl-MSO the concentration of active inhibitory isomer was about 25 % of that shown.